*Indian J. Weed Sci.* **40** (3 & 4) : 101-108 (2008)

# Molecular Diversity of Little Seed Canary Grass (*Phalaris minor* Retz.) Populations from Wheat Growing Belts of India

**Rupa S. Dhawan[1], Rohtas Singh[2], S. S. Punia[1], A. K. Dhawan[2] and S. S. Dudeja[3]**
CCS HAU Regional Research Station, Uchani, Karnal-132 001 (Haryana), India

## ABSTRACT

Random amplified polymorphic DNA analysis (RAPD) was conducted on isoproturon susceptible and resistant *Phalaris minor* populations from different wheat growing states in India for assessment of population diversity. Nine isoproturon susceptible populations from different wheat growing locations in India and 10 resistant populations from different locations in Punjab and Haryana states were analysed. Eleven random primers were used for amplification of DNA. Dissimilarity values amongst biotypes ranged from 0.049 to 0.95 indicating high level of heterogeneity among these populations. Cluster tree analysis grouped the biotypes into three major groups. The resistant biotypes were observed in all the three groups indicating the evolution of resistance to isoproturon in the three groups independently. Some of the populations showed resemblance with each other by amplification of common fragments indicating their common origin and spread to other areas possibly by cultivation practices and crop seed.

**Key words :** RAPD analysis, genetic variability, herbicide resistance evolution, *P. minor*



## INTRODUCTION

*Phalaris minor* Retz. is a self pollinating, annual, grassy weed infesting winter crops especially wheat in almost all the wheat growing states of India viz., Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, Bihar and Jammu and Kashmir. Isoproturon [N (4-isopropyl-phenyl) N, N' dimethyl urea], a phenylurea herbicide has been used to control this weed since 1970's (Bhan *et al*., 1976; Gill *et al*., 1978). Isoproturon resistant populations have been observed after their continuous use for over 15 years in Haryana and Punjab in an area where rice has been rotated with wheat. This resulted in drastic reductions in wheat yields (Malik and Singh, 1995; Walia *et al*., 1997). Although reported from a small area initially isoproturon resistant biotypes are now spread to major parts of Haryana and Punjab. Isoproturon is still found effective and is in use in some of the areas in Punjab and other states in India. The mechanism of evolution of resistance to isoproturon was reported to be due to enhanced degradation of the herbicide in a population from Haryana (Singh *et al*., 1998 a, b). Alternate herbicides like diclofop, tralkoxydim and pendimethalin provided control of the resistant biotypes initially for 1-2 years but could not be recommended for long term use because of their inconsistent performance (Malik *et al*., 1995). Herbicides like fenoxaprop, clodinafop and sulfosulfuron were recommended in 1998 for the control of IPU resistant *P. minor* biotypes. Poor efficacy of fenoxaprop was reported from some locations within three years of recommendation (Yadav *et al*., 2001). The causes for poor efficacy of isoproturon have variously been attributed to be faulty spray techniques, spurious herbicide formulations, misapplication timing and rates of herbicides and unfavourable weather conditions. It was considered of interest, to study, if biological variability within *P. minor* populations was a cause for variable efficacy of the herbicides at different locations.

Diversity within weed populations has generally been estimated by differences in morphological parameters wherever apparent and correlated with herbicide efficacy. *Cardebra* species have been seen to show differential sensitivity to 2, 4-D (Mullighan and Findlay, 1974). Protein profiles provided indications regarding variability in *Echinocloa crusgalli* populations from different habitats (Gasquez and Compoint, 1976). Isoenzyme analysis also provided indications regarding genetic diversity in *Echinocloa* species from Spain (Asins *et al*., 1999). Recently, PCR (Polymerase Chain Reaction) based methods are being employed to make assessments regarding genetic diversity within weed

[1]Department of Agronomy, CCS HAU, Hisar.
[2]Centre for Plant Biotechnology, Department of Science and Technology, Govt. of Haryana, Hisar.
[3]Department of Microbiology, CCS HAU, Hisar.

species. Genetic variability in *Senecio vulgaris* (Muller-Scharer and Fischer, 2001) and in *Elytrigia repens* (Mitra *et al*., 2000) has been established by RAPD analysis. A differential sensitivity of *Echinocloa* species has been correlated with quinclorac efficacy (Lopez-Martinez *et al*., 1999). In another study on *E. crusgalli* in Arkansas, multiple origins of propanil resistance was established by RAPD analysis indicating that simple control of weed seed dispersal would not stop the spread of resistance (Rutledge *et al*., 2000). RAPD data have also been useful to assess the influence of herbicide application on genetic variation in *Sinapis arvensis* L. (Moodie *et al.,* 1997) and characterize genetic shifts in *Poa annua* caused by selection pressure from herbicides (Sweeney and Dannerberger, 1995).

*P. minor* being a self pollinating species, not much variation was expected within or amongst populations. A preliminary effort regarding assessment of genetic diversity in isoproturon susceptible (S) and resistant (R) *P. minor* populations by RAPD analysis indicated some differences between few populations tested from within Haryana (Singh *et al*., 2004; Dhawan *et al*., 2005; McRoberts *et al*., 2005). In this study, assessment regarding genetic diversity among populations from different wheat growing states of India has been made by RAPD analysis. The objectives of the present study was (i) to assess levels of heterogeneity within S populations from different wheat growing belts of India, (ii) to assess levels of heterogeneity as affected by evolution of resistance to herbicides from different regions in Haryana and Punjab (iii) to identify markers for the resistant trait if possible and (iv) to provide leads regarding mode of origin and spread of R populations.

## MATERIALS AND METHODS

Seeds of 19 biotypes *of P. minor* were collected in April 2005. These included seeds of isoproturon S biotypes from Jabalpur (S1), Gwalior (S2), Uttaranchal (S3), Palampur (S4), Faizabad (S6), Samastipur (S7), Ranchi (S8), Karnal (S9) and Hisar (S10) and of putative isoproturon R biotypes from Ludhiana (R1),


Fig. 1. Map showing approximate distribution of *P. minor* sampling sites in India. Each number corresponds to a distinct population. Numbers preceded by S represent susceptible populations and those preceded by R represent resistant populations.

Amritsar (R2), Sangrur (R3), Kapurthala (R4), Uchana (R5), Sagga (R6), Hisar – Dabra (R7), Bhiwani (R8), Ambala (R9) and Kaithal (R10) (Fig. 1). Each collection consisted of seed from approximately 50 plants at random, pooled together and kept in plastic bottles at room temperatures in the laboratory. The seed of R biotypes was tested for isoproturon resistance in a pot bioassay and were found to possess a resistance factor of 4-6. These were allowed to germinate after six months in Petri dishes in a growth chamber maintained at 18 ± 2 $^{O}$C with 9 h photoperiod. After one week of seedling growth, 100 mg of the leaf tissue in duplicate (upper 2-3 leaf tips from approximately10 seedlings) was taken for RAPD analysis. The tissue was washed thoroughly with mild detergent and then with water. It was then rinsed in 70% alcohol, followed by 3-4 washings with double distilled water to remove the traces of alcohol. It was ground to a fine powder in liquid nitrogen. Leaf DNA was extracted using the kit developed by Bangalore Genie (Bangalore-560 058, India; Cat No.105547) and stored at – 20 $^{O}$C until used. The precipitated DNA was finally solubilized in 20 µL of solution B in the kit. The DNA concentration was estimated by measuring absorbance at 260 nm with a UV spectrophotometer and samples diluted to obtain 50 ng/µL concentration. The quality of DNA was tested by agarose (1.4%) gel electrophoresis. For RAPD analysis, the protocol described by Williams *et al*. (1990), for PCR amplification and agarose electrophoresis was employed with slight modifications. Eleven random primers were employed for the study. The primer sequence is given in Table 1. These primers had been tested for amplification of DNA in *P. minor* in earlier studies (Singh *et al*., 2004; Dhawan *et al.,* 2005).

PCR mix was prepared for RAPD reaction mixture in a volume of 25 uL in an Eppendorf tube containing 0.5 uL *Taq* polymerase (15 unit), 2.5 uL of 10 X assay buffer with 15 mM $MgCl_2$, 1.0 uL mix dNTP's- deoxynucleotide triphosphates ( 2.0 mM each); 1.0 uL primer ( 50-60 ng/mL) and 1.0 µL sample DNA (50 ng/uL). The mixture was spun for 5 sec and kept in the thermocycler for amplification of DNA. Temperatures for different steps for amplifications had already been standardized (Dhawan *et al*., 2005) and were as follows. The amplification consisted of an initial denaturation for 6 min at 94 $^{O}$C, 40 cycles of denaturation for 1 min at 94 $^{O}$C, annealing at 42 $^{O}$C for 2 min and extension for 1 min at 72 $^{O}$C. After the final cycle, the samples were incubated for 5 min at 72 $^{O}$C and then kept at 4 $^{O}$C. One reagent control that included all the components of the PCR reaction except sample DNA was made to monitor any possible contamination. Samples were loaded in the 1.4% agarose gel containing ethidium bromide (0.5 mg/ml). Electrophoresis was carried out in Tris-EDTA buffer. Each assay was repeated twice with similar results.

PCR products were viewed under UV light using photodyne UV illuminator. Molecular weight was estimated by using 1 kb DNA ladder. Each band was scored independently as 1 for presence and 0 for absence. The percentage of polymorphic bands was calculated as the proportion of polymorphic bands over the total number of bands. Dendrogram was generated by UPGMA method using the NT SYS –PC version computer software.

Table 1. List of 11 primers and their sequences that produced polymorphic bands among *Phalaris minor* populations

| Reference code | Forward sequence | No. of markers | No. of polymorphic markers |
|---|---|---|---|
| KJ1 | 5' GAA ACG GGT G 3' | 9 | 9 |
| KJ2 | 5' GTG ATC GCA G 3' | 7 | 7 |
| KJ3 | 5' TCG GCG ATA G 3' | 9 | 9 |
| KJ4 | 5' GTC CAC ACG G 3' | 7 | 6 |
| KJ5 | 5' CTG CTG GGA C 3' | 8 | 8 |
| KJ6 | 5' GTA GAC CCG T 3' | 8 | 8 |
| KJ7 | 5' GGG CCG TTT A 3' | 10 | 10 |
| KJ8 | 5' CCA CCA ACA G 3' | 8 | 8 |
| KJ9 | 5' TTG AGA CAG G 3' | 7 | 7 |
| KJ10 | 5' TTC GAC CAT C 3' | 5 | 5 |
| KJ11 | 5' CCT ACC TTA TTG ACT GCA ACT TCT 3' | 5 | 5 |
| Total | | 83 | 82 |

Table 2. Proportion of polymorphic markers per primer by RAPD markers from nine S populations of *P. minor* sampled from different wheat growing areas in India and 10 R populations sampled from different areas in Haryana and Punjab

| Primer | S1 | S2 | S3 | S4 | S6 | S7 | S8 | S9 | S10 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KJ1 | 0 | 0 | 0.4 | 0.8 | 1 | 0.4 | 0.9 | 0.3 | 0.1 | 0.2 | 0.2 | 0 | 0.4 | 0.7 | 0 | 0 | 0.4 | 0.4 | 0 | 0.3 |
| KJ2 | 0 | 0 | 0 | 0.3 | 0 | 0.6 | 0.6 | 0.7 | 0.6 | 0 | 0.6 | 0 | 0.6 | 0 | 0 | 0.6 | 0.9 | 1 | 0 | 0.3 |
| KJ3 | 0.3 | 0.2 | 0.2 | 0.8 | 0.4 | 0.4 | 0.8 | 0.8 | 0.2 | 0.6 | 0.8 | 0.4 | 0.4 | 0.8 | 0.2 | 0.4 | 1 | 1 | 0.4 | 0.5 |
| KJ4 | 0.4 | 0.4 | 0.4 | 0.7 | 0.4 | 0.7 | 0.7 | 0.4 | 0.6 | 0 | 0.9 | 0.4 | 0.4 | 0.7 | 0.4 | 0.9 | 0.9 | 0.9 | 0.9 | 0.6 |
| KJ5 | 0.6 | 0.1 | 0.1 | 0.8 | 0.6 | 0.8 | 0.8 | 0.8 | 0.8 | 0 | 0.8 | 0.8 | 0.8 | 0.8 | 0 | 0 | 1 | 1 | 0.8 | 0.6 |
| KJ6 | 0.3 | 0 | 0 | 0.4 | 0.3 | 0.3 | 0.4 | 0 | 0.1 | 0 | 0.3 | 0.3 | 0.3 | 0.4 | 0 | 0.3 | 1 | 1 | 0 | 0.3 |
| KJ7 | 0 | 0 | 0 | 1 | 0.3 | 0.3 | 1 | 0.2 | 0.2 | 0 | 0.6 | 0.2 | 0.2 | 0.3 | 0 | 0 | 1 | 1 | 0 | 0.3 |
| KJ8 | 0.5 | 0.6 | 0.6 | 0.9 | 0.9 | 0.9 | 0.9 | 0.9 | 0.5 | 0.1 | 0.9 | 0.5 | 0.8 | 0.8 | 0 | 0.6 | 0.9 | 0.9 | 0 | 0.6 |
| KJ9 | 0 | 0 | 0 | 0.6 | 0 | 0.6 | 0.6 | 0 | 0 | 0 | 0.3 | 0 | 0 | 0 | 0 | 0 | 1.3 | 0.6 | 0 | 0.2 |
| KJ10 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0.5 | 0.8 | 1 | 0 | 0.2 |
| KJ11 | 0 | 0 | 0.3 | 0.3 | 0.3 | 0.5 | 1 | 0.5 | 0.3 | 0 | 0.3 | 0 | 0.8 | 0.3 | 0.3 | 0.3 | 1 | 1 | 0.3 | 0.4 |
| Mean | 0.2 | 0.1 | 0.2 | 0.6 | 0.4 | 0.5 | 0.7 | 0.4 | 0.3 | 0.1 | 0.5 | 0.2 | 0.4 | 0.5 | 0.1 | 0.3 | 0.9 | 0.9 | 0.2 | 0.4 |

Table 3. Dissimilarity index (1-F) amongst isoproturon S and R populations of *Phalaris minor* based on simple matching coefficients

| | S1 | S2 | S3 | S4 | S6 | S7 | S8 | S9 | S10 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | R9 | R10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 0 | 0.2 | 0.4 | 0.7 | 0.3 | 0.5 | 0.7 | 0.5 | 0.5 | 0.4 | 0.6 | 0.1 | 0.4 | 0.5 | 0.4 | 0.4 | 0.7 | 0.7 | 0.3 |
| S2 | | 0 | 0.2 | 0.8 | 0.5 | 0.7 | 0.8 | 0.5 | 0.5 | 0.3 | 0.8 | 0.3 | 0.6 | 0.6 | 0.3 | 0.5 | 0.8 | 0.8 | 0.3 |
| S3 | | | 0 | 0.6 | 0.3 | 0.5 | 0.7 | 0.4 | 0.4 | 0.4 | 0.6 | 0.5 | 0.4 | 0.4 | 0.3 | 0.5 | 0.7 | 0.7 | 0.3 |
| S4 | | | | 0 | 0.4 | 0.2 | 0.1 | 0.4 | 0.5 | 0.8 | 0.2 | 0.6 | 0.4 | 0.3 | 0.8 | 0.5 | 0.3 | 0.3 | 0.7 |
| S6 | | | | | 0 | 0.3 | 0.5 | 0.3 | 0.4 | 0.6 | 0.4 | 0.3 | 0.2 | 0.2 | 0.5 | 0.5 | 0.6 | 0.6 | 0.4 |
| S7 | | | | | | 0 | 0.2 | 0.2 | 0.3 | 0.7 | 0.3 | 0.5 | 0.2 | 0.4 | 0.7 | 0.5 | 0.3 | 0.3 | 0.6 |
| S8 | | | | | | | 0 | 0.4 | 0.5 | 0.8 | 0.2 | 0.6 | 0.4 | 0.4 | 0.9 | 0.5 | 0.2 | 0.2 | 0.7 |
| S9 | | | | | | | | 0 | 0.3 | 0.5 | 0.4 | 0.5 | 0.2 | 0.4 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 |
| S10 | | | | | | | | | 0 | 0.6 | 0.4 | 0.5 | 0.3 | 0.4 | 0.6 | 0.5 | 0.5 | 0.6 | 0.5 |
| R1 | | | | | | | | | | 0 | 0.8 | 0.5 | 0.6 | 0.6 | 0.4 | 0.6 | 0.8 | 0.8 | 0.4 |
| R2 | | | | | | | | | | | 0 | 0.6 | 0.4 | 0.3 | 0.8 | 0.4 | 0.3 | 0.3 | 0.7 |
| R3 | | | | | | | | | | | | 0 | 0.3 | 0.4 | 0.5 | 0.5 | 0.7 | 0.7 | 0.4 |
| R4 | | | | | | | | | | | | | 0 | 0.3 | 0.6 | 0.4 | 0.4 | 0.4 | 0.5 |
| R5 | | | | | | | | | | | | | | 0 | 0.6 | 0.4 | 0.4 | 0.4 | 0.5 |
| R6 | | | | | | | | | | | | | | | 0 | 0.4 | 0.9 | 0.9 | 0.1 |
| R7 | | | | | | | | | | | | | | | | 0 | 0.5 | 0.6 | 0.5 |
| R8 | | | | | | | | | | | | | | | | | 0 | 0 | 0.7 |
| R9 | | | | | | | | | | | | | | | | | | 0 | 0.7 |
| R10 | | | | | | | | | | | | | | | | | | | 0 |



## RESULTS AND DISCUSSION

All the primers produced polymorphic markers. A total of 83 different markers were produced. Out of these one was monomorphic and 82 polymorphic. The total number of markers amplified per primer varied between 5-10 (Table 1). The highest proportion of polymorphic markers was produced by KJ4 and KJ8 (0.6) averaged over populations followed by KJ3 (0.5). KJ9 and KJ10 produced lesser (0.2) polymorphic markers. The highest incidence of polymorphism occurred in Ranchi (S8) population amongst the S biotypes where 70% of markers were polymorphic. Amongst the R biotypes highest incidence of polymorphism (90%) was seen in Bhiwani (R8) and Ambala (R9). The range of polymorphism was 10-90% amongst biotypes (Table 2). Dissimilarity values of 19 *P. minor* biotypes (both R and S) revealed genetic diversity relationship among them (Table 3). The dissimilarity values ranged from 0.0 to 0.90. A high

level of polymorphism observed among *P. minor* biotypes from different geographical locations in India is indicative of the fact that the populations are heterogeneous. This high level of heterogeneity could be one of the factors for variable response to the herbicides. A large interpopulation variability has been observed amongst other self pollinating species like *Echinocloa* and *Avena* species (Andrews *et al*., 1998; Asins *et al*., 1999). Within population diversity in these weeds was low owing to the strict autogamous behaviour of the weeds. McRoberts *et al*. (2005) reported high polymorphic values within seedlings from a single earhead of *P. minor* employing Inter Simple Sequence Repeat (ISSR) analysis indicating possibility of outcrossing within the species. In a strictly self pollinating species the seed from a single earhead should be alike. Some more studies using PCR based molecular markers revealed that self fertilizing species had high genetic diversity within a population in addition to inter population diversity and suggested the possibility of higher outcrossing rates than previously assumed (Green *et al.,* 2001; Muller–Scharer and Fischer, 2001). Matus and Hucl (1999) reported high level of polymorphism in *P. minor* as compared to other species like *P. canariensis*, *P. brachystachys, P. paradoxa* and *P. angusta* by isoenzyme anlaysis.This was attributed to its tetraploid chromosomal complement as compared to other *Phalaris* species which are reported to be diploid (Andersen, 1961). Enzyme electrophoresis has demonstrated that auto tetraploids maintain high level of heterozygosity than their diploid progenitors by tetrasomic inheritance (Crawford, 1989). Multiple herbicide resistance reported in *P. minor* recently (Chhokar and Sharma, 2008) is possibly a consequence of the high heterogeneity observed in this weed. This also points towards possibility of evolution of multiple resistance mechanisms adopted by the weed to combat herbicide selection pressure. Molecular techniques involving gene sequencing could reveal the kind of mutants evolved that would warrant management. Least dissimilarity (0.0) was seen between the populations from Bhiwani (R8) and Ambala (R9) within Haryana which are 200 km apart. This was followed by a dissimilarity value of 0.1 seen between biotypes from Sagga (R6) and Kaithal (10) within Haryana (30 km apart) from Jabalpur (S1) and Sangrur (R3) more than 1000 km apart and between biotypes from Palampur (S4) and Ranchi (S8) more than 1500 km apart. Little genetic distance between R populations from Bhiwani and Ambala (0.0) and from Sagga and Kaithal (0.1) from within Haryana may indicate that they may have originated from a single population and spread by movement of seed either due to cultivation practices, camal irrigation water or through crop seed (Table 3, Fig. 2). The close genetic association of geographically distant S populations may be due to dispersal in crop seed prior to evolution of isoproturon resistance. Wheat seed lots contaminated with *P. minor* regularly traded among farmers could cause this (Yaduraju, 1999). Cluster analysis grouped the 19 populations in three main clusters (Fig. 2). Groups I and II included both S and R biotypes and Group III included only R biotypes. The genetic distance between Groups I and II was 0.30 and genetic distance with Group III was 0.55. Group I included S biotypes from Jabalpur, Gwalior, Uttaranchal and Hisar as also the R biotypes from Sangrur, Ludhiana, Sagga, Kaithal and Hisar–Dabra. Similarly, Group II included S biotypes from Palampur,

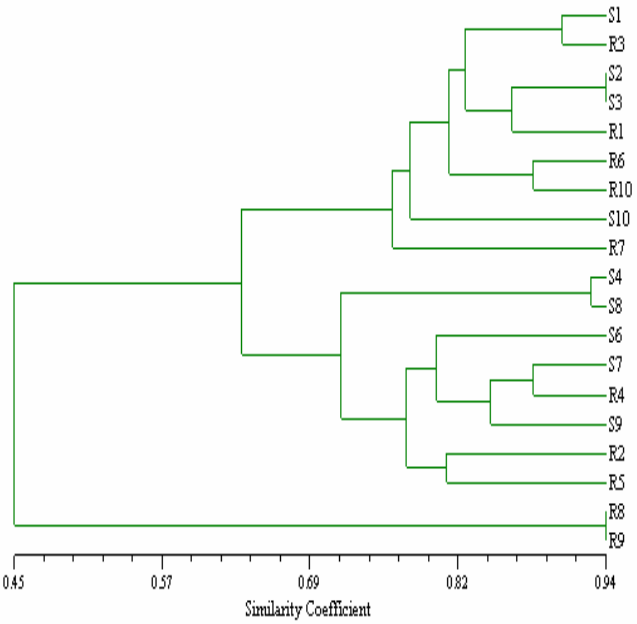


Fig. 2. Dendrogram showing genetic relationship between S and R biotypes of *P. minor* based on RAPD marker analysis. Group I consists of S biotypes from Jabalpur, Gwalior, Uttaranchal and Hisar-Farm and R biotypes from Sangrur, Ludhiana, Sagga, Kaithal and Hisar–Dabra. Group II consists of S biotypes from Palampur, Ranchi, Faizabad, Samastipur and Karnal – Uchani, and R biotypes from Kapurthala, Amritsar and Karnal–Uchana. Group III consists of only two R biotypes from Ambala and Bhiwani.

Ranchi, Faizabad, Samastipur and Karnal–Uchani and R biotypes from Kapurthala, Amritsar and Karnal–Uchana. Group III included only R biotypes from Ambala and Bhiwani. Presence of some common DNA fragments exclusively in S biotypes of group II and their absence in S biotypes of group I and of some more DNA fragments exclusively in the R biotypes in group III is an indication of three broad categories of biotypes. The R biotypes probably evolved independently in the three groups (Tables 4 and 5). It is apparent from the presence of both S and R biotypes from Hisar in group I and those of S and R biotypes from Karnal both from Haryana in group II.

This study therefore reveals high levels of heterogeneity within *P. minor* populations which could be responsible for variable efficacy of different herbicides at different locations. The level of heterogeneity was not affected by evolution of herbicide resistance as observed in other weeds like *Alopecurus myosuroides* and *Chenopodium album* (Warwick and Marriage, 1982; Chauvel and Gasquez, 1994). In weeds like *Amaranthus* and *Brassica rapa* it was reported to be reduced as a result of herbicide selection pressure (Warwick and Black, 1980; Warwick and Black, 1993). Local evolution of herbicide resistance appears to be the main cause for the appearance of R biotypes. However, as with other weeds, seed movement also played an important role in the spread of R populations. Andrews *et al*. (1998) concluded that seed movement played an important role in the spread of herbicide R *Avena fatua* L. Stankiewiez *et al*. (2001) also reported that seed movement was important in the spread of *Solanum nigrum* L. across Europe. In contrast Cavan *et al*. (1998) concluded local evolution was most important in the evolution of herbicide R *A. myosuroides* Huds on farms in the United Kingdom.



## ACKNOWLEDGEMENTS

This study was supported by a grant from Indian Council of Agricultural Research, New Delhi in the form of an ad-hoc research project. Seed material supplied by different AICRP-WC centres is gratefully acknowledged. Thanks are also due to Dr. N. T. Yaduraju, former Director, NRCWS, Jabalpur for his keen interest in the project.

Table 4. DNA fragments exclusively amplified by isoproturon S and R biotypes

| Primer | Base pair ( bp) | Biotypes |
|---|---|---|
| | | **Susceptible** |
| KJ1 | 500 | Faizabad |
| KJ11 | 550 | Ranchi |
| KJ4 | 250,300 | Group II (Palampur, Faizabad, Samastipur, Ranchi, Uchani) |
| KJ8 | 800,900 | Group II (Palampur, Faizabad, Samastipur, Ranchi, Uchani) |
| | | **Resistant** |
| KJ4 | 500 | Amritsar, Uchana, Hisar (Dabra), Kaithal |
| KJ10 | 300 | Amritsar, Uchana, Bhiwani, Ambala, Hisar (Dawra) |
| KJ2 | 500 | Group III ( Ambala, Bhiwani) |
| KJ2 | 450 | Group III ( Ambala, Bhiwani) |
| KJ3 | >1000 | Group III ( Ambala, Bhiwani) |
| KJ3 | 1000 | Group III ( Ambala, Bhiwani) |
| KJ5 | 800 | Group III ( Ambala, Bhiwani) |
| KJ5 | 700 | Group III ( Ambala, Bhiwani) |
| KJ6 | 900 | Group III ( Ambala, Bhiwani) |
| KJ6 | 700 | Group III ( Ambala, Bhiwani) |
| KJ6 | 600 | Group III ( Ambala, Bhiwani) |
| KJ6 | 500 | Group III ( Ambala, Bhiwani) |
| KJ6 | 450 | Group III ( Ambala, Bhiwani) |
| KJ10 | 450 | Group III ( Ambala, Bhiwani) |
| KJ11 | 700 | Group III ( Ambala, Bhiwani) |

Table 5. DNA fragments shared by some isoproturon S and R biotypes

| Primer | Base pair (bp) | Biotypes |
|---|---|---|
| | | **Susceptible and resistant** |
| KJ7 | 800 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 700 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 600 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 550 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 500 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 400 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 350 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 300 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 250 | S–Ranchi, Palampur; R–Ambala, Bhiwani |
| KJ7 | 200 | S–Ranchi, Palampur; R–Ambala, Bhiwani |



## REFERENCES


Andersen, D. E. 1961. Taxonomy and distribution of the genus *Phalaris*. *Iowa State. J. Sci.* **36** : 1-96.

Andrews, T. S., I. M. Morrison, G. A. Penner, 1998. Monitoring the spread of ACCase inhibitor resistance among wild oat *(Avena fatua*) patches using AFLP analysis. *Weed Sci.* **46** : 196-198.

Asins, M. J., J. L. Carretero, A. Del Best, E. A. Carbenell and D. G. DeBarreda, 1999. Morphologic and isoenzyme variation in barnyard grass (*Echinocloa*) weed species. *Weed Technol*. **13** : 200-215.

Bhan, V. M., P. S. Negi and G. R. Singh., 1976. Effect of metoxuron on the control of *P. minor* Retz. in wheat and its residual effect on subsequent crops. *Ind. J. Weed Sci*. **8** : 70-73.

Cavan, G., P. Biss and S. R Moss, 1998. Localized origins of herbicide resistance in *Alopecurus myosuroides*. *Weed Res.* **38** : 239-245.

Chauvel, B. and J. Gasquez, 1994. Relationships between genetic polymorphism and herbicide resistance within *Alopecurus myosuroides* Huds. *Heredity* **72** : 336-344.

Chhokar, R. S. and R. K. Sharma. 2008. Multiple herbicide resistance in little seed canary grass (*Phalaris minor*) : A threat to wheat production in India. *Weed Biol. and Manage*. **8** : 112-123.

Crawford, D. J. 1989. Enzyme electrophoresis and plant systematics. p 146-164. In : *Isozymes in Plant Biology,* D. E. Soltis and P. S. Soltis (eds.). Dioscorides Press, Portland, O. R.

Dhawan, R. S., A. K. Dhawan, S. Kajla and R. Moudgil, 2005. Assessment of variation in isoproturon susceptible and resistant biotypes of *Phalaris minor* Retz. by RAPD analysis. *Ind. J. Biotechnol*. **4** : 534-537.

Gasquez, J. and J. P. Compoint, 1976. Apport de l'e'lectrophorese en courant pulse'a'la tazonomie d *Echinocloa crusgalli* (L). P. B. *Ann. Amelior. Plantes* **26** : 345-355.

Gill, H. S., U. S.Walia and L. S. Brar, 1978. Control of *Phalaris minor* Retz. and wild oats in wheat with new herbicides. *Pesticides* **12** : 55-56.

Green, J. M., J. H. A. Barker, E. J. P. Marshall, R. J. Froud Williams, N. C. B. Peters, G. M. Arnold, K. Dawson and A. Karp, 2001. Microsatellite analysis of the inbreeding grass weed Barren Brome (*Anisantha sterilis*) reveals genetic diversity at the within and between farm scales. *Mol. Ecol*. **10** : 1035-1045.

Lopez-Martinez, N., A. P. Salva, R. P. Finch and R. De Prado, 1999. Molecular markers indicate intraspecific variation in the control of *Echinocloa* sp. with quinclorac. *Weed Sci.* **47** : 310-315.

Malik, R. K., A. Yadav, V. K. Garg, R. S. Balyan, Y. S. Malik, S. Singh and R. Dhawan, 1995. Herbicide resistance : Current status and research findings. Extension Year Bulletin. CCS HAU, Hisar, India. pp. 37.

Malik, R. K. and S. Singh, 1995. Little seed canary grass (*Phalaris minor*) resistance to isoproturon in India. *Weed Technol*. **9** : 419-425.

Matus, M. and P. Hucl, 1999. Isoenzyme variation within and among accessions of Annual *Phalaris* species in North American germplasm collections. *Crop Sci*. **39** : 1222-1228.

McRoberts, N., W. Sinclair, A. Mcpherson, A. C. Franke, R. P. Saharan, R. K. Malik, S. Singh and G. Marshall, 2005. An assessment of genetic diversity within and between populations of *Phalaris minor* using ISSR markers. *Weed Res*. **45** : 431-439.

Mitra, S., P. C. Bhowmik and A. Idnurum, 2000. Using RAPD markers to identify genetic variation in quackgrass (*Elytrigia repens*) biotypes. *Ann. Appl. Biol.* **136** : 253-258.

Moodie, M., R. P. Finch and G. Marshall, 1997. Analysis of genetic variation in wild mustard (*Sinapis arvensis*) using molecular markers. *Weed Sci*. **45** : 102-107.

Muller-Scharer, H. and M. Fischer, 2001. Genetic structure of the annual weed *Senecio vulgaris* in relation to habitat type and population size. *Mol. Ecol*. **10** : 17-28.

Mullighan, G. A. and J. N. Findlay, 1974. The biology of Canadian weeds. 3. *Cardaria draba, C. chalpensis* and *C. pubescens*. *Can . J. Plant Sci*. **54** : 149-160.

Rutledge, J., R. E. Talbert and C. H. Sneller, 2000. RAPD analysis of genetic variation among propanil resistant and susceptible *Echinocloa crusgalli* populations in Arkansas. *Weed Sci*. **48** : 669-674.

Singh, D. V., A. K. Gaur and D. P. Mishra, 2004. Biochemical and molecular mechanisms of resistance against isoproturon in *Phalaris minor*. Variations in protein and RAPD profiles of isoproturon resistant and sensitive *Phalaris minor* biotypes. *Ind. J. Weed Sci.* **36** : 256-259.

Singh, S., R. C. Kirkwood and G. Marshall, 1998a. Effect of ABT on the activity and rate of degradation of isoproturon in susceptible and resistant biotypes of *Phalaris minor* and in wheat. *Pestic. Sci.* **53** : 123-132.

Singh, S., R. C. Kirkwood and G. Marshall, 1998b. Effect of the monoxygenase inhibitor piperonyl butoxide on the herbicidal activity and metabolism of isoproturon in herbicide resistant and susceptible biotypes of *P. minor*

and wheat. *Pestic. Biochem. Physiol.* **59** : 143-153.

Stankiewiez, M., G. Gasamski and S. Gawronski, 2001. Genetic variation and phylogenetic relationships of triazine-resistant and triazine susceptible biotypes of *Solanum nigrum* analysis using RAPD markers. *Weed Res.* **41** : 287-300.

Sweeney, P. M. and T. K. Dannerberger, 1995. RAPD characterization of *Poa annua*. Populations in golf course greens and fairways. *Crop Sci*. **37** : 1595-1600.

Walia, S. S., L. S. Brar and B. K. Dhaliwal, 1997. Resistance to isoproturon in *Phalaris minor* Retz. in Punjab. *Pl. Prot. Quarterly* **12** : 138-140.

Warwick, S. I. and L. D. Black, 1986. Electrophoretic variation in triazine-resistant and susceptible populations of *Amaranthus retroflexus* L. *New Phytol*. **104** : 661-670.

Warwick, S. I. and L. D. Black, 1993. Electrophoretic variation in triazine-resistant and susceptible populations of the allogamous weed *Brassica rapa*. *Weed Res.* **33 :** 105-114.

Warwick, S. I. and P. B. Marriage, 1982. Geographical variation in populations of *Chenopodium album* resistant and susceptible to atrazine. I. Between and within population variation in growth and response to atrazine. *Canad. J. Bot*. **60** : 483-493.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalaski and S. V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res.* **18** : 6531-6535.

Yadav, A., R. K. Malik, K. D. Sayre, L. S. Brar and G. Singh, 2001. Cross resistance in *Phalaris minor* against fenoxapropred signals appear in India. Abstract. In : Proc. 1st Biennial Conference in the New Millenium on Eco-friendly Weed Management Options for Sustainable Agriculture, organized by ISWS and UAS, Bangalore.

Yaduraju, N. T. 1999. Control of herbicide resistant *Phalaris minor* need for a sound weed management system. *Pestology* **23** : 264-272.